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# Heterogeneity of plasma low-density lipoproteins and atherosclerosis risk

**Ronald M. Krauss**

Life Sciences Division, Lawrence Berkeley Laboratory, University of California, Berkeley, California, USA

Increased levels of IDL and small, dense LDL are associated with the risk of coronary artery disease. Possible mechanisms include increased susceptibility of small, dense LDL to oxidation, and to other pathologic effects, such as increased retention in the arterial wall. Beneficial effects of a low-fat diet and certain lipid-lowering therapies on the levels and properties of small, dense LDL or their precursors may contribute substantially to the reductions in coronary atherosclerosis observed in several lipid-lowering trials.

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## Introduction

Apolipoprotein (apo)B-containing lipoprotein particles exhibit a considerable variation in their physical and chemical properties that may affect their metabolism and potential for involvement in atherogenesis. Because of the strong evidence for direct involvement of LDL and IDL in the development of atherosclerotic lesions, most attention has focused on the pathologic properties of these classes of particles. Since the initial review of this topic in this journal in 1991 [1], more information has become available regarding specific biochemical and metabolic features of the IDL and LDL subpopulations that may relate to atherogenesis. In addition, specific dietary, hormonal, and pharmacologic effects on the subclass profiles have indicated the need to consider lipoprotein heterogeneity when defining the mechanisms of interventions designed to lower the risk of developing coronary disease.

## Physicochemical and metabolic heterogeneity of low-density lipoprotein and intermediate-density lipoprotein

LDL and IDL subpopulations have been defined on the basis of a number of characteristics, including particle density, size, charge, and lipid and apolipoprotein content [2,3]. The distribution of mass among the LDL subclasses in plasma is generally reflected by the particle diameter and buoyant density of the predominant LDL species [2]. Particle diameter is mostly assessed by nondenaturing gradient-gel electrophoresis, which can identify as many as seven distinct electrophoretic components based on variations in particle size and shape. Density gradient and analytical ultracentrifugation are used to assess LDL buoyant density and flotation rate ($S_f^0$), respectively. Numerous studies have demonstrated strong correlations between plasma triglyceride and VLDL levels, and increasing density and decreasing size of the predominant LDL species. In turn, these LDL characteristics are inversely related to the levels of plasma HDL, particularly the $HDL_2$ subclass [4,5]. At present no detailed understanding of the metabolic basis for these relationships exists. Available evidence suggests that heparin-releasable lipase activities may be intimately involved. Postheparin plasma lipoprotein lipase (LPL) activity is associated with increased levels of larger LDL [6] and $HDL_2$ [7], and this may be caused by, at least in part, the transfer of surface lipids and apolipoproteins during chylomicron and VLDL-triglyceride hydrolysis. Conversely, factors contributing to increased plasma triglyceride levels can promote triglyceride enrichment of larger LDL or IDL particles that give rise to smaller, denser products, presumably through the action of hepatic lipase, which is inversely associated with the buoyancy of LDL [8•]. Similar processes may contribute to the conversion of the larger $HDL_2$ species to smaller HDL particles.

Although these metabolic factors appear to be important overall determinants of the LDL particle distribution, available evidence also implies that major aspects

**Abbreviations**

**apo**—apolipoprotein; **CAD**—coronary artery disease; **CETP**—cholesteryl ester transfer protein; ***d***—density; **LPL**—lipoprotein lipase; **$S_f^0$**—flotation rate.

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of LDL heterogeneity result from the production of differing forms of LDL from different VLDL and IDL precursors. At least two distinct IDL subspecies have been identified in normal humans [3] that appear to be precursors of large (LDL-I) and intermediate (LDL-II) LDL subclasses, respectively [9]. Parallel processing of particles in these two pathways by triglyceride enrichment and lipolysis may then give rise to smaller, denser species (LDL-III and LDL-IV). Two major LDL production pathways have been demonstrated in the pig, and evidence for a genetic determinant of the selective overproduction of the larger, more buoyant species of LDL, in a spontaneously hypercholesterolemic strain of pigs, has been presented by Aiello *et al.* [10•].

In addition, evidence is available that the activity of cholesteryl ester transfer protein (CETP) influences the distribution of the LDL subclasses. In patients with homozygous familial CETP deficiency, accumulation of a smaller, triglyceride-rich LDL subpopulation is found with a density distribution that overlaps with the predominant, larger LDL species [11]. A similar phenomenon occurs in individuals with an alcohol-induced reduction in CETP activity. Following alcohol withdrawal, the particle distribution becomes more homogeneous and the levels of CETP normalize [12]. Recent evidence has indicated that a primary acceptor for CETP-mediated HDL cholesteryl ester transfer in normolipidemic individuals is a large, buoyant, triglyceride-enriched LDL subclass [13•]. Thus, it is possible that retention of such triglyceride-rich LDL and subsequent lipolytic processing contributes to the heterogeneous LDL subclass profile found in patients with CETP deficiency.

It is not known to what extent differences in receptor-mediated clearance of the LDL subclasses contribute to variations in the plasma LDL particle distribution. Relatively reduced LDL receptor binding has been reported for more buoyant and dense LDL, compared with the intermediate-density LDL subspecies [14]. Interestingly, in a patient homozygous for familial defective $apoB_{100}$, dense LDL particles [density ($d$) > 1.040 g/ml] accumulate preferentially in plasma [15•]. This is caused by a lack of binding of these particles to the LDL receptor, whereas mid-density LDL ($d$ 1.034–1.040 g/ml) appear to retain some receptor binding. The most buoyant LDL species ($d$ < 1.034 g/ml) undergo LDL receptor-mediated clearance by virtue of an increased content of apoE. When apoE-containing particles are removed by immunoabsorption, this fraction, like dense LDL, is completely deficient in receptor binding. The reduction in receptor-binding affinity of the smaller, denser LDL found in hypertriglyceridemic individuals has been shown recently to be independent of triglyceride content [16•]. Furthermore, triglyceride enrichment of LDL does not appear to affect conformation of apoB in the receptor-binding domain [17]. Thus, the basis for reduced receptor interaction of $apoB_{100}$ in large and small, but not intermediate LDL remains to be determined.

Differences in nonreceptor-mediated LDL clearance among the LDL subpopulations may also contribute to variations in the LDL particle distribution. Binding to arterial wall proteoglycans [18] and uptake by aortic subendothelial cells [19] occurred at a higher rate for LDL with a reduced sialic acid content. Because smaller, denser LDL particles are depleted in sialic acid [20], their retention in arterial tissue and subsequent modification, possibly by oxidative processes, may contribute to their plasma clearance.

## Small, dense, low-density lipoprotein phenotype

A distinct LDL subclass pattern characterized by a predominance of small, dense LDL particles (principally LDL-III) has been identified using both nondenaturing gradient-gel electrophoresis [21] and analytic ultracentrifugation [2] (Miller BD *et al.*, unpublished data). This trait, which has been designated LDL subclass pattern B, is found in 30–35% of adult men, but is much lower in men less than 20 years of age and in premenopausal women (5–10%) [21,22], with a slightly higher prevalence (15–25%) in postmenopausal women [22,23••]. Evidence from several studies for a major gene determinant of this phenotype has been summarized previously by Austin in 1992 [24]. More recently, two studies [25,26], using complex segregation analysis, have confirmed major gene effects on LDL diameter as a quantitative trait. The data have been most consistent with either an autosomal dominant or codominant model for inheritance of the pattern B phenotype with varying additive and polygenic effects. Linkage of pattern B to the region of the LDL receptor gene locus on chromosome 19p [27] has been confirmed, using quantitative sib-pair linkage analysis of LDL particle size, in 25 kindreds selected on the basis of having two affected family members with coronary artery disease (CAD) [28]. In these families, preliminary evidence suggested a linkage of pattern B to regions near three other genetic loci: the apoA-I/C-III/A-IV cluster on chromosome 11, the CETP locus on chromosome 16, and the manganese superoxide dismutase gene on chromosome 6. Thus, multiple genes may contribute to the determination of particle size of the major LDL subclass in plasma, and the responsible genetic mechanisms may differ among affected families.

Estimates of heritability of LDL particle size range from approximately 30–50% [24], indicating the importance of nongenetic and environmental influences. In view of the close relationship between the change in plasma triglyceride levels with alterations in LDL particle size [5,29] and the other metabolic relationships described above, it is likely that both genetic and nongenetic determinants of pattern B confer coordinate effects on plasma triglyceride and LDL subclass metabolism. In addition to age and gender, effects on LDL particle size and density distribution have been shown with abdominal adiposity [30], oral contraceptive use [31•], and

hypertriglyceridemia occurring with acquired immunodeficiency syndrome [32•]. As will be discussed, variations in dietary fat and carbohydrate can also strongly influence the expression of the small, dense LDL phenotype, and contribute to variations in LDL particle size distribution that are observed in individuals and population groups.

Another important metabolic correlate of the pattern B lipoprotein profile is insulin resistance, manifest as relative elevations in fasting and postglucose insulin levels [23••,33••], fasting glucose [34•], and increased steady-state plasma glucose levels during a constant insulin infusion [33••]. The metabolic syndrome associated with insulin resistance includes raised triglyceride and reduced HDL levels, and the relationships of these variables to LDL particle size appears to account for the correlation of smaller LDL diameter with parameters of insulin resistance [33••]. Insulin resistance has also been related to increased blood pressure, and this can be demonstrated in pattern B individuals with this syndrome [23••,33••]. A high prevalence of the small, dense LDL trait has been found in patients with non-insulin-dependent diabetes mellitus [35,36], and it is likely that this reflects the insulin resistance found in the majority of patients with this disease. In addition, a relative increase in LDL-III, in conjunction with increased levels of IDL, has been reported [37] in patients with well-controlled insulin-dependent diabetes mellitus.

Thus, the occurrence of the small, dense LDL phenotype may result from the interaction of multiple genetic and environmental determinants, and the trait can be viewed as a marker for the mechanisms underlying these effects. In particular, the prevalence of pattern B characteristically denotes triglyceride levels greater than 140–160 mg/dl, and is rarely found in association with triglyceride levels below 100–110 mg/dl [21]. Possible candidate mechanisms include those that result in overproduction or impaired clearance of triglyceride-rich lipoproteins, or both. A reduced activity of LPL has been found in individuals expressing the pattern B trait [38], and patients with heterozygous LPL deficiency have a lipoprotein phenotype that appears identical to that described in pattern B individuals [39••]. In these individuals, reduced exogenous triglyceride clearance is independent of the fasting triglyceride level, suggesting that one or more factors responsible for retardation of triglyceride-rich lipoprotein metabolism may have an etiologic or contributory role in a high proportion of individuals with the small, dense LDL phenotype. A hypothetical scheme for the production of this phenotype is shown in Figure 1.

## Low-density lipoprotein heterogeneity and risk of coronary artery disease

The plasma lipoprotein profile accompanying a predominance of small, dense LDL particles (specifically LDL-III) is associated with up to a threefold increase in the susceptibility of developing CAD. This has been demonstrated in case-control studies of myocardial infarction [40] and angiographically documented coronary disease [41,42,43••]. In all studies to date, the disease risk associated with small, dense LDL is no longer signifi-

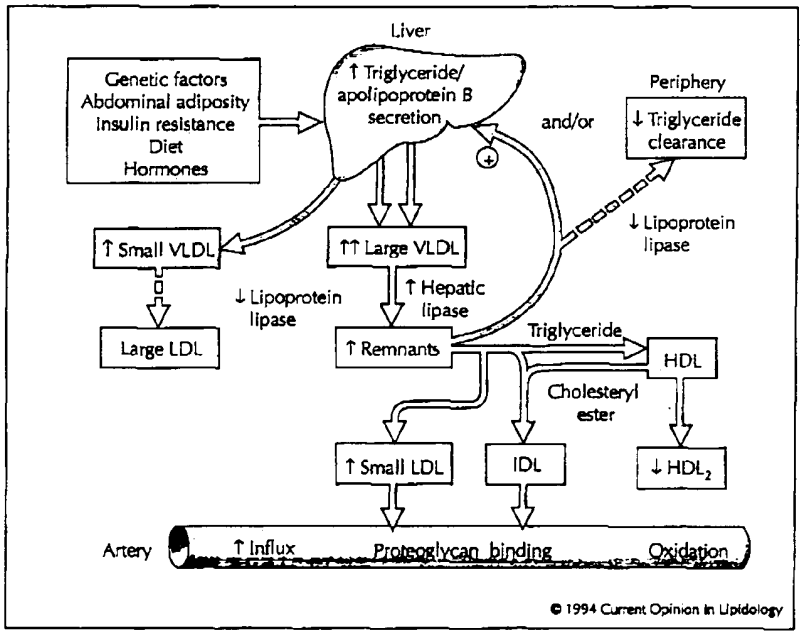


**Fig. 1.** Hypothetical scheme for atherogenic lipoprotein phenotype. Genetic and environment factors may promote increased production or reduced clearance of triglyceride-rich lipoproteins, or both. Increased triglyceride production leads to an increased secretion of large VLDL particles. Larger VLDL, as well as smaller VLDL (which give rise to larger LDL particles), may accumulate owing to reduced lipoprotein lipase activity. Reduced peripheral clearance can result in diversion of lipolytic remnants to the liver where their uptake may further stimulate VLDL secretion. Remnants not cleared in the liver are modified by exposure to hepatic lipase, leading to the formation of small LDL. Finally, increased plasma-residence time of remnants may promote lipid exchange with HDL, resulting in the formation of cholesterol-enriched IDL and acceleration of $HDL_2$ catabolism. Small LDL and IDL may promote atherosclerosis as a result of increased entry and retention in the arterial wall and increased susceptibility to oxidation modification. ↑—increase; ↓—decrease.

cant after adjusting for triglyceride [40,41,43••] or other risk factors, including LDL and HDL cholesterol [42]. A number of limitations are found in cross-sectional comparisons, however, including difficulties in adjusting statistically for the effects of strongly interrelated variables, such as plasma triglyceride, HDL, and LDL particle size.

Much stronger conclusions may be drawn from prospective or longitudinal studies in which changes in disease status are correlated with variations in candidate risk factors, such as levels of triglyceride-rich lipoproteins and HDL. Among the numerous prospective studies of the angiographic progression of CAD that have been carried out to date, several have measured the lipoprotein subfractions. In the National Heart, Lung, and Blood Institute Type II Coronary Disease Intervention Trial [44], lipoprotein subfractions measured by analytical ultracentrifugation were correlated with the angiographic progression of coronary disease in patients with primary hypercholesterolemia treated with cholestyramine or diet, or both [44]. The progression status was most closely related to changes in IDL mass, as measured by analytic ultracentrifugation ($P<0.03$), and less strongly to a change in mass of small, dense LDL of $S_f°$ 0–7. In the recent St Thomas' Angiographic Regression Study [45••], changes in coronary segment lumen diameter were assessed by quantitative coronary angiography in 74 men treated with cholestyramine or diet, or both. Among a number of lipid and lipoprotein subfraction measures, those that correlated most closely with increases in lumen diameter were the on-trial levels of cholesterol in IDL, LDL-II ($d=1.019$–$1.040$ g/ml), LDL-III ($d=1.040$–$1.063$ g/ml) and $HDL_3$ (inverse correlation). Multiple linear regression analysis revealed that only LDL-III cholesterol correlated significantly and independently with both of the measures of segment lumen diameter employed in this study. Angiographically assessed coronary disease progression in a third recent study [46••] was most strongly correlated with changes in cholesterol levels in the VLDL remnants and IDL, with much weaker relationships for cholesterol and apoB in LDL. Preliminary information regarding the LDL subclasses and coronary disease progression has also been reported from the Stanford Coronary Risk Intervention Project [47]. This project was a multiple risk factor intervention trial studying patients with angiographically documented coronary disease, in whom the most commonly used regimens included bile acid binding resins and nicotinic acid [48]. Despite similar levels of total LDL cholesterol at time of entry into the trial, and similar reductions with therapy, only the patients with predominantly small, dense LDL, and not those with larger, more buoyant LDL, demonstrated reduced angiographic progression compared with the control groups [47]. It is noteworthy that a post-hoc analysis of the results of the Helsinki Heart Trial [49] indicated that the major benefit of diet plus gemfibrozil on the incidence of myocardial infarction and cardiac death was confined to only 10% of the patients with triglyceride levels greater than 204 mg/dl and LDL : HDL cholesterol ratios greater than five, a subgroup that would be expected to consist primarily, if not exclusively, of patients with predominantly smaller, denser LDL particles. Moreover, a recent post-hoc analysis of the results of the Cholesterol-Lowering Atherosclerosis Study [50] has revealed that the benefit of intervention with diet, colestipol, and nicotinic acid on coronary disease progression was confined to individuals in the top third of the triglyceride distribution (> 190 mg/dl), a group expected to consist mainly of LDL subclass pattern B individuals.

Thus, in various reports, both IDL and small, dense LDL have been associated with clinical and angiographic indices of CAD. Given the relationships of these lipoprotein subclasses with each other, and potentially with other unmeasured pathologic factors, these studies do not allow for assessment of causality. Nevertheless, it is reasonable to suppose that one or both of these subclasses directly contribute to the risk of developing CAD, particularly in individuals with LDL subclass pattern B, in whom levels of both IDL and small, dense LDL are elevated. As will be discussed, it is possible that IDL and dense LDL particles promote different pathologic events in the development of atherosclerotic cardiovascular disease, or that they share common features resulting in additive or overlapping effects on this process. In animal models, particularly nonhuman primates, large cholesteryl-ester-rich, apoE-containing IDL-like particles are most closely correlated with the extent of coronary atherosclerosis [51]. The atherogenicity of these particles has been related to an increased content of cholesteryl oleate, as compared with cholesteryl linoleate [52], possibly leading to differences in the physical state of the LDL particle core. Large, cholesteryl-ester-rich LDL have also been documented in spontaneously hypercholesterolemic pigs with a predisposition to developing CAD [53]. In other animal models of diet-induced atherosclerosis, such as rabbits and nonhuman primates as well as in human dysbetalipoproteinemia, increased levels of cholesterol-rich β-VLDL were clearly atherogenic. It is possible that cholesterol-enriched remnants and IDL in humans have pathologic properties similar to both β-VLDL and large apoE-containing LDL. The atherosclerosis risk is increased in individuals who accumulate these particles as a result of dietary and genetic factors, including predisposition to the small, dense LDL phenotype. At present, an animal homolog of this phenotype has not been identified.

It is noteworthy that a predominance of small, dense LDL is commonly found in conjunction with familial disorders of lipoprotein metabolism associated with an increased risk of premature CAD. These include familial combined hyperlipidemia [54], hyperapobetalipoproteinemia [55], and hypoalphalipoproteinemia [56]. Thus, it is possible that a portion of the coronary disease risk in these syndromes is mediated by the metabolic changes found in conjunction with LDL subclass pattern B.

## In-vitro studies of atherogenic properties of low-density lipoprotein subfractions

A number of studies have documented that LDL subfractions differ in susceptibility to in-vitro oxidative stress [57,58,59•,60•]. Specifically, large, buoyant LDL are more resistant, and small, dense LDL are more susceptible to oxidation, as assessed by the length of the lag period between copper incubation and the propagation phase of free-radical generation. A number of factors have been proposed to contribute to this differential susceptibility, including increased content of polyunsaturated fatty acids [57], altered properties of the surface lipid monolayer [61••], possibly associated with reduced content of free cholesterol [58], and diminished content of antioxidants, particularly ubiquinol-10 [61••]. Reaven *et al.* [62••] showed that dense LDL fractions are preferentially protected from copper-induced oxidation when the diet is enriched in monounsaturated, but not polyunsaturated, fat in patients taking vitamin E. These observations, coupled with the recent findings comparing the oxidative depletion of surface-localized parinaric acid with core-localized parinaric acid methyl ester (Tribble DL *et al.*, unpublished data) have led to the concept that the increased potential for lipids in smaller, denser LDL particles to undergo peroxidation *in vitro* may be caused by the reduced protection conferred by the surface lipid monolayer. This, in turn, may be related to reduced free cholesterol and antioxidant content.

As described above, reduced LDL sialic acid content, which is a feature of smaller, denser LDL particles [20], results in a greater affinity for arterial wall proteoglycans [18], and a greater capacity to promote enrichment of aortic subendothelial cells with cholesteryl ester [19]. Other studies [63•–65•,66,67] have identified differences among the LDL subfractions in their effects on cellular processes that might adversely affect vascular function. Compared with more buoyant particles, LDL fractions (*d* 1.033–1.045 g/ml) showed greater stimulation of thromboxane synthesis by human umbilical vein endothelial cells [63•], and LDL-III (*d* 1.040–1.045 g/ml) resulted in greater intracellular free calcium accumulation in cultured rat aortic smooth muscle cells [64•]. In another recent report [65•], a denser LDL subfraction (*d* 1.039–1.063 g/ml) was associated with a tissue protein factor inhibitor with anticoagulant activity. This finding would be expected to confer an antiatherosclerotic effect on dense LDL; however, the authors speculate that oxidation of dense LDL in the artery wall could neutralize the anticoagulant effect of this inhibitor. The LDL-III subfraction has reduced LDL receptor-mediated catabolism by human monocyte-derived macrophages [66]. This finding is consistent with evidence discussed earlier that denser LDL has a reduced binding affinity for fibrobast LDL receptors, and it raises the possibility that the uptake of these particles, in their native form, by macrophages in the arterial wall is reduced. Finally, Dyce *et al.* [67] reported recently that the LDL cholesterol : plasma apoB ratio, as an index of LDL particle size and density, is strongly related to endothelial vasodilator dysfunction in patients with coronary disease, and is independent of other lipoprotein variables.

## Effects of lipid-lowering therapies on low-density lipoprotein subfractions

### Diet and exercise

Cross-sectional population analyses [68] have suggested an association between reduced LDL particle size and relatively reduced dietary animal-fat intake, and increased consumption of carbohydrates. Dietary effects on LDL subclass levels have been evaluated recently in a study [69•] of 105 healthy middle-aged men who each consumed a high-fat diet (46% fat, 34% carbohydrate) and a low-fat diet (24% fat, 56% carbohydrate) for 6 weeks in a randomized crossover design. Polyunsaturated- and saturated-fat diets were reduced in parallel to achieve a constant polyunsaturated : saturated ratio (0.7), and the intake of monounsaturated fat, cholesterol, protein, and dietary fiber were similar in the two diets. Men with a predominance of small, dense LDL (pattern B) on the high-fat diet (n = 18) exhibited a twofold greater reduction in LDL cholesterol than men with the pattern A phenotype. Furthermore, only pattern B individuals showed significant reductions in plasma apoB and LDL relative to HDL-cholesterol levels. Out of the 87 men with pattern A on the high-fat diet, 36 converted to pattern B on the low-fat diet. The group differences in LDL and apoB responses could not be attributed to differences in plasma lipid levels, body mass index or to apoE phenotypes. Taken together, these results indicate that in the majority of men, the reduction in LDL cholesterol seen on a low-fat, high-carbohydrate diet is mainly because of a shift from larger, more cholesterol-enriched LDL to smaller, cholesterol-depleted LDL. Much greater reductions in LDL cholesterol, as well as reduced LDL particle number, are achieved in individuals with a predominance of small, dense LDL on a high-fat diet.

Changes in the fatty-acid composition of the diet can also have important effects on LDL structure, composition, and metabolism. Compared with dietary enrichment with saturated fat (lard), polyunsaturated fat (corn oil) has been shown, in guinea pigs, to result in a preferential reduction and enhanced receptor-mediated clearance of larger LDL particles compared with smaller, denser LDL [70]. This may occur as a result of up-regulation of apoB, E receptors with a greater affinity for larger, rather than smaller LDL particles. Although polyunsaturated fat feeding reduces coronary artery atherosclerosis in African green monkeys, and the extent of atherosclerosis is positively related to LDL particle size, no mean reduction of LDL size was found with unsaturated- versus saturated-fat diets in this species [71•].

In cynomolgus monkeys, dietary fish oil, when compared with lard, resulted in a reduced size of LDL in association with a reduced cholesteryl ester and apoE content and reduced LDL receptor binding [72]. Although a similar reduction in LDL cholesteryl ester content was previously observed in normal humans consuming omega-3 fatty acids compared with corn oil, no differences were found in LDL size or receptor binding [73]. However, in patients with mixed hyperlipidemia, treatment with 3 g/day of omega-3 fatty acids resulted in a significant increase in LDL particle diameter in conjunction with reduced triglyceride levels [74], which is consistent with a change in LDL-subclass distribution.

Exercise training has also been shown to reduce the mass of small, dense LDL, with a shift toward larger, more buoyant and cholesterol-enriched LDL particles [75–77]. These effects appear to be mediated largely by exercise-induced reductions in adiposity, because similar increases in particle diameter were observed with an equivalent amount of diet-induced weight loss in overweight individuals [76].

### Hormonal factors

Although, as noted above, the small, dense LDL phenotype is substantially higher in postmenopausal than premenopausal women, estrogen replacement therapy in healthy postmenopausal women results in a reduced mass of large, buoyant LDL particles, and a shift toward intermediate-sized LDL subspecies [78•,79•]. In dyslipidemic postmenopausal women, with predominantly smaller LDL particles, estrogen replacement resulted in reduced plasma LDL cholesterol and apoB concentrations without a change in LDL size [80], which indicated a reduction in mass of the smaller LDL species. These results suggest that estrogen deficiency alone is not a cause of the increased prevalence of the small LDL phenotype in postmenopausal women, and imply that estrogen replacement therapy can be beneficial in reducing the LDL mass in women who manifest this trait. As noted above, oral contraceptive use in younger women is associated with a significant shift in mass from LDL-I to LDL-III compared with nonusers, and this difference is independent of other lipid and lipoprotein variables [31•]. This could result from effects of the more potent estrogens used in oral contraceptives; however, it is more likely to be related to progestational effects, possibly including increased hepatic lipase activity. Recently, a predominance of small, dense LDL has been reported in the last trimester of pregnancy, with a reversion to a larger LDL profile postpartum [81], again suggesting an important interaction of sex steroid hormones with factors regulating the LDL particle distribution.

### Pharmacologic agents

A number of studies have investigated the effects of lipid-lowering therapies on LDL heterogeneity. In general, the findings conform to previous evidence that variations in the LDL size and density profile are closely related to variations in plasma triglyceride levels. Shifts from smaller, more dense to larger, more buoyant LDL have been observed in conjunction with lipid lowering in hypertriglyceridemic patients treated with nicotinic acid [82], acipimox, a nicotinic acid derivative [83], clofibrate [84•], gemfibrozil [85,86], and ciprofibrate [87••]. In the latter study, complete normalization of the mass distribution, composition, and size of LDL subspecies in patients with combined hyperlipidemia was achieved together with a 33% lowering of plasma triglyceride levels. In contrast, despite a 55% reduction of plasma triglyceride with gemfibrozil treatment in familial combined hyperlipidemic patients, the LDL peak-particle diameter remained small, and the dense LDL mass remained elevated in relation to control subjects [88•]. An increase in the net LDL flotation rate was observed, but this was primarily associated with an increased mass of buoyant LDL. It has been reported [89••] that the reduction of plasma triglyceride and LDL cholesterol in hypercholesterolemic patients treated with fenofibrate was associated with a shift in LDL input from a pool with a slow turnover to a more rapidly cleared pool. Thus, the effectiveness of fibrate therapy in normalizing the LDL subclass distribution may depend on the extent to which treatment induces differences in the production of larger, more rapidly cleared LDL in relation to the formation of smaller LDL. In addition, the absolute degree of triglyceride elevation [82], and the nature of the underlying genetic and metabolic abnormalities affecting triglyceride metabolism may be important determinants of drug-induced changes on the LDL subclass profile.

In studies of the effects of β-hydroxy-β-methylglutaryl-coenzyme A reductase inhibitors, increases in LDL particle size have been described for fluvastatin [85], lovastatin [90], and simvastatin [91,92••,93] generally in relation to lowering both plasma triglyceride and VLDL levels. In several studies [74,94,95] involving another β-hydroxy-β-methylglutaryl-coenzyme A reductase inhibitor, pravastatin, no significant changes in LDL size or LDL subclass phenotype were observed, which was possibly related to insignificant reductions in plasma triglyceride levels in these studies. Gaw *et al.* [92••] found a preferential increase in the clearance of larger, VLDL-derived LDL-I and LDL-II, induced by simvastatin, presumably resulting from increased apoB, E receptor activity. These results were similar to the effects previously reported for bile-acid sequestrant therapy [96]. In addition, probucol treatment of hypercholesterolemic patients resulted in a selective reduction of larger, more buoyant LDL particles, as well as in the triglyceride enrichment of these particles in conjunction with increased plasma CETP activity [97].

In patients with CAD, the use of beta-blockers was associated with a significantly lower LDL peak flotation rate, indicating a shift to smaller LDL particles [98]. This was primarily due to significantly lower levels of

larger LDL ($S_f^o$ 7–12), with nonsignificant increases of smaller LDL ($S_f^o$ 0–7). These findings suggest that, as in the study of gemfibrozil noted above, changes in mass of the larger LDL, but not smaller LDL, may in some instances underlie changes in the LDL particle profile.

## Conclusion

The physical, chemical, and metabolic properties of the subpopulations within the IDL- and LDL-particle spectrum have important influences on mechanisms underlying atherosclerotic cardiovascular disease. Of particular interest have been the triglyceride-rich lipoprotein remnants and IDL that, in human and animal studies, show consistent relationships with the development and progression of atherosclerosis. In addition, increased levels of smaller, denser LDL, principally LDL-III, signify a common atherogenic metabolic phenotype strongly related to disordered triglyceride metabolism, and to features of the insulin resistance syndrome. A number of case-control studies have corroborated an increase in the risk of coronary atherosclerosis and myocardial infarction with a predominance of smaller LDL. Moreover, increased levels of small, dense LDL have been found in a majority of the familial disorders that have a high risk of developing premature CAD, which is consistent with the genetic influences underlying this trait. Some properties of the small LDL particles, notably enhanced susceptibility to oxidative stress and interactions with arterial wall components, suggest that biological mechanisms may be responsible for their preferential involvement in the pathogenesis of coronary disease. It is possible, however, that the increased coronary risk results from the combined effects of multiple metabolic changes associated with the small LDL phenotype. A number of therapeutic interventions in humans, including a low-fat diet and various lipid-lowering drugs, can effectively reduce elevated levels of small, dense LDL, although a concomitant reduction in the coronary disease risk in a prospective trial has not been documented. Of particular interest is the possibility that some treatments may preferentially benefit individuals with the small LDL trait and, thus, the efficacy of coronary disease risk reduction in the general population can be improved further by the application of individualized therapeutic guidelines.

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Ronald M. Krauss, Life Sciences Division, Lawrence Berkeley Laboratory, University of California, 1 Cyclotron Road, Berkeley, CA 94720, USA.